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Laser Desorption/Ionization Time-of-Flight Mass Spectrometry on Sol−Gel-Derived 2,5-Dihydroxybenzoic Acid Film

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This work presents a novel method for direct desorption/ionization of analytes from sol−gel-derived film. 2,5-Dihydroxy benzoic acid (DHB), a common MALDI matrix, was incorporated into a sol−gel polymeric structure. The sol−gel-derived DHB thin film can assist the mass analysis of analytes by laser desorption/ionization, with a matrix interference-free background in the mass spectra. The sol−gel-derived film can function as an energy absorber during laser irradiation because it contains DHB molecules. Furthermore, laser irradiation with normal laser power (70−110 μJ) is not likely to generate any background ions from this sol−gel-DHB derived film. The samples were prepared straightforwardly. After a thin film was formed on a Parafilm membrane from the sol−gel-derived DHB solution coating, the sample solution was directly added to the top of the film, for laser desorption/ionization mass analysis. The analyte signals were homogeneously obtained on the sol−gel-derived DHB film. Experimental results show that the optimum concentrations of DHB incorporated in the sol−gel solution were between 7500 ppm and 10 000 ppm, providing a matrix interference-free background. Analytes, including small proteins, peptides, amino acids, and small organics, were used to demonstrate the effectiveness of the proposed method. However, a higher laser power (>110 μJ) than normal was required to desorb small proteins from the sol−gel-derived DHB film. Therefore, a few matrix ions desorbed from the thin film were generated during laser irradiation. The detection limit for both small molecules and proteins, using this sol−gel-assisted laser desorption/ionization (SGALDI) mass spectrometry (MS), was as low as 81 fmol. However, a mass spectrometer with cutoff-mass selection could detect 8.1 fmol of cytochrome *c*. The largest analyte observed by the SGALDI-MS in this study was myoglobin.

Since its development,[1,2] matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) has been extensively used in various fields of research.[3−6] However, analyzing low-mass molecules using conventional MALDI mass spectrometry is difficult, because the unavoidable matrix interference appears in the low-mass range. Several alternative matrix systems have been developed to solve this problem.[7−28] Mixing an inorganic powder, such as carbon or metal, with glycerol to use as a matrix has reduced matrix background.[7−19] Matrix-free methods, such as desorption/ionization on porous silicon (DIOS) facilitated by electrochemically treating silicon surfaces,[20−24]and mass analysis, using silicon films formed from a silicon surface by using plasma-enhanced chemical vapor deposition, have recently been successfully applied to the analysis of small organics.[25] Additionally,

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previous studies have demonstrated the feasibility of using a film substrate to support samples for laser desorption/ionization mass analysis. [19, 26~28].

This study proposes a novel method, called sol−gel-assisted laser desorption/ionization (SGALDI) mass spectrometry, which uses an organic/inorganic hybrid film as the sample substrate to generate matrix interference-free mass spectra. The film is aimed to function like the MALDI matrix but without contributing ions to the background in the mass spectra. Analytes with low mass can be easily identified under such conditions. This goal can be achieved by rigidly binding sufficient laser light absorber molecules to a polymeric structure. The modified polymer can then become an absorber of the laser energy used in MALDI. The laser power normally used to generate analyte ions during laser irradiation is insufficient to desorb the entrapped molecules that are covalently bonded to the polymeric structure. A matrix interference-free background is thus obtained. This study demonstrates the feasibility of incorporating sufficient MALDI matrixes and UV light absorber molecules into a high molecular polymeric film to meet this goal.

A thin film was constructed using the sol−gel technique, which involves the processes of hydrolysis, condensation, and polymerization, [29] usually carried out at room temperature. Both inorganic and organic dopants are easily doped into the sol−gel solution to generate a hybrid sol−gel polymer. Molecules with hydroxyl or amine groups can covalently bond to the polymeric structure during the sol−gel processes.[30] The characteristics of a thin film made by sol−gel processes are easily altered by adding dopants. 2, 5-Dihydroxybenzoic acid (DHB), a common MALDI matrix, was selected as a dopant to be added to the sol−gel solution to change the properties of the sol−gel product. The two hydroxyl functional groups attached to the aromatic ring of DHB are ideal binding-sites at which to incorporate DHB into the sol−gel structure. DHB can naturally bind with the products of the hydrolysis of the sol−gel precursor, such as tetraethoxysilane (TEOS), in the condensation and polymerization steps of the sol−gel processes. The sol−gel product generated only by TEOS does not have any capacity to absorb the laser energy at the 337-nm wavelength used in MALDI. However, once sufficient DHB molecules are doped into the sol−gel structure, this sol−gel derivative is expected to become a DHB-like material for MALDI analysis. Desorbing the trapped DHB from the high molecular polymeric film during laser irradiation is difficult since DHB covalently bonds to the net structure of the sol−gel polymer. A higher laser power may be required to break the covalent bonds between the entrapped DHB and the polymeric structure than is normally required to desorb free DHB molecules. Accordingly, this approach can generate a matrix interference-free background in the mass spectra under normal power of laser irradiation for generating analyte signals.

EXPERIMENTAL SECTION

Phenylalanine, arginine, bradykinin, insulin, cytochrome *c*, and DHB were obtained from Sigma (St. Louis, MO). Prometryn, decyltrimethylammonium bromide, dodecyltrimethylammonium bromide, and hexadecyltrimethylammonium bromide were obtained from Riedel-de Haën (Deisenhofen, Germany). TEOS was purchased from Acros, while hydrochloric acid was purchased from Merck (Darmstadt, Germany).

The sol−gel derivative was prepared by reacting various amounts of DHB (0.12, 0.09, 0.06, 0.045, and 0.03 g) in a starting sol solution, which consisted of 4.5 mL of TEOS (MW 208.33, density 0.934), 1 mL of water, and 0.5 mL of 0.01 N hydrochloric acid. The mixture was stirred for ~1 day before use. A 0.1-μL sample of this sol−gel-derived DHB solution was then applied to a Parafilm membrane (American National Can, WI), which had already been attached to a sample plate using double-sided black carbon tape (Ted Pella). The sol−gel product was very difficult to wash off from the surface of the sample plate after it had been directly deposited on it. The Parafilm was used as the substrate for the sol−gel film on the sample plate to prevent this problem. After a thin film was formed (taking ~20 min) on the Parafilm membrane, the sample solution was deposited on the top of the membrane. The sample was ready for mass analysis after the volatile solvent had evaporated. The sol−gel-derived DHB solution was stored at −20 °C in a refrigerator whenever it was not immediately used. Generally, the sol−gel-derived DHB solutions (≤10 000 ppm) lasted for 2 weeks, without any DHB-derived ions being observed in the mass spectra in the analysis of the low-mass organics.

The laser power generally used to generate analytes with molecular sizes less than 5000 Da was in the range of 70−110 μJ without showing any matrix background signals in the SGALDI mass spectra. The laser focus was ~200 μm^2. If laser power larger than 110 μJ was used in SGALDI analysis, background ions derived from DHB molecules were observed in the SGALDI mass spectra. However, analytes such as cytochrome *c* generally required laser power larger than 110 μJ for effective desorption/ionization in SGALDI analysis. Thus, it was not surprising to observe the matrix ions along with the cytochrome *c* ions revealed in the SGALDI mass spectra. According to our experiments, the laser power required to successfully desorb small proteins such as cytochrome *c* in MALDI analysis was about 70−90 μJ. That is, the laser power used in SGALDI analysis is slightly higher than that in MALDI analysis.

The experiments were performed usinga a Biflex III (Bruker) linear time-of-flight mass spectrometer. The mass spectrometer was equipped with a 337-nm nitrogen laser, a 1.25-m flight, and a sample target with the capacity to load 384 samples simultaneously. The accelerating voltage was set to 19 kV.

RESULTS AND DISCUSSION

Figure 1 illustrates the sol−gel processes that use TEOS as the precursor material and in which DHB is added to the sol solution as the dopant. DHB is expected to be able to covalently bind to the products of the hydrolysis of TEOS and to become part of the sol−gel-derived polymer. Figure 2 shows the proposed polymeric structure formed by incorporating DHB into the sol−gel polymeric structure. The sol−gel-derived DHB film may be able to assist the desorption/ionization of analytes during laser irradiation. The capacity may well depend on the number of DHB incorporated in the film. Various concentrations of DHB were incorporated in the sol−gel solution, and coatings of the product

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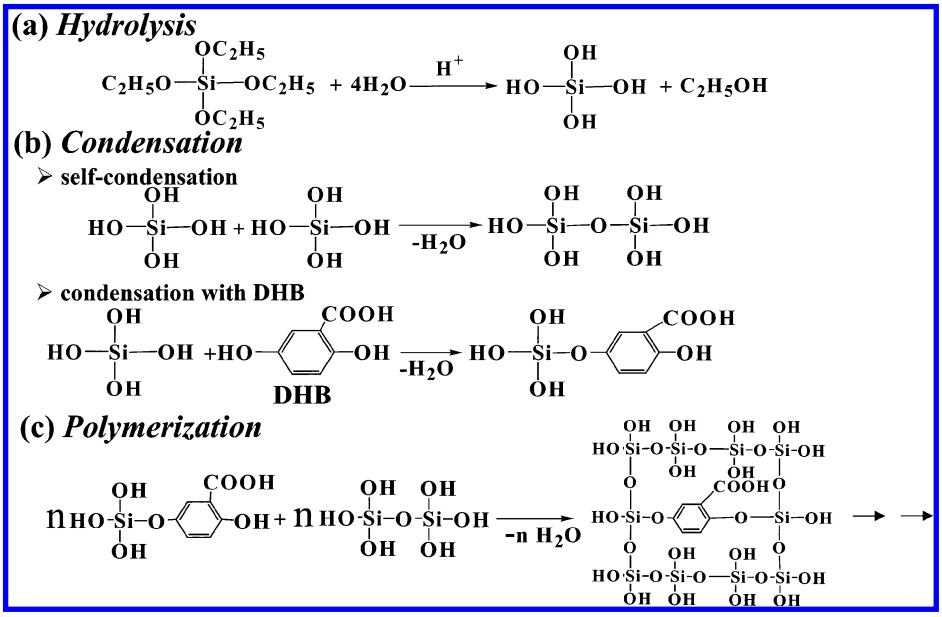

Figure 1. Three steps of the sol−gel processes. TEOS was used as the precursor material.

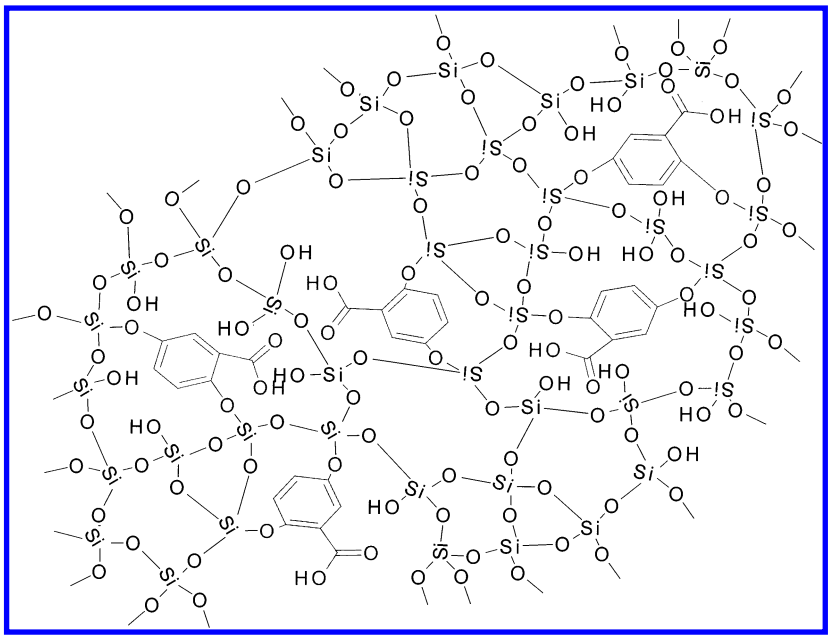

Figure 2. Proposed polymeric structure for entrapping DHB into the sol−gel polymer.

on a Parafilm membrane were used as the MALDI matrix for desorption/ionization mass analysis to prove this assumption.

Figure 3 shows the laser desorption mass spectra of prometryn desorbed from the sol−gel-derived DHB film with various trapped concentrations (5000, 7500, 10 000, 15 000, and 20 000 ppm) of DHB. Clearly, the prometryn signal increases with the concentration of DHB in the sol−gel-derived film. The same laser power was used in all cases shown in Figure 3. The mass spectra in Figure 3a−c show no ions in the background, except prometryn quasimolecular ions. However, a few ions derived from DHB appear at m/z = 137 and 155 in the mass spectra in Figure 3d and e. All the DHB molecules seem to have covalently bonded to the polymeric structure, when the concentrations of DHB were below 10 000 ppm in the sol−gel-derived DHB film. Thus, no DHB-derived ions were observed in the low-mass region. However, when excess DHB molecules (above 10 000 ppm) were present in the sol−gel-derived film, the free DHB molecules were very easily desorbed by laser irradiation. A matrix interference-free background can be obtained using this approach when the concentrations of DHB in the sol−gel film are carefully adjusted to an appropriate range. The experimental results show that the concentrations of DHB in the sol−gel-derived film used to generate a matrix interference-free background were below 10 000 ppm. However, if insufficient DHB molecules are incorporated in the sol−gel-derived film used in the analysis, the direct laser desorption/ionization analytes from this film may be difficult. The optimum concentrations of DHB entrapped in the sol−gel-derived film with a matrix interference-free background are between 7500 and 10 000 ppm.

Figure 4 shows the laser desorption/ionization mass spectrum of the mixture that contains equal amounts (10 ng) of decyltrimethylammonium bromide, dodecyltrimethylammonium bromide,

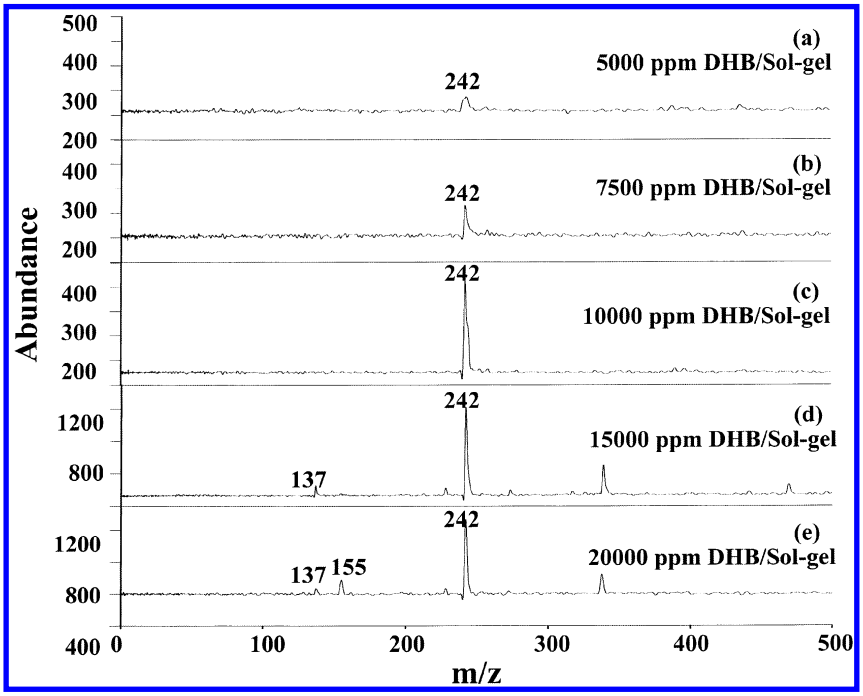

Figure 3. Laser desorption/ionization mass spectra of prometryn (MW 241.2, 100 ng) desorbed from the sol−gel-derived DHB films, which contained (a) 5000, (b) 7500, (c) 10 000, (d) 15 000, and (e) 20 000 ppm of DHB.

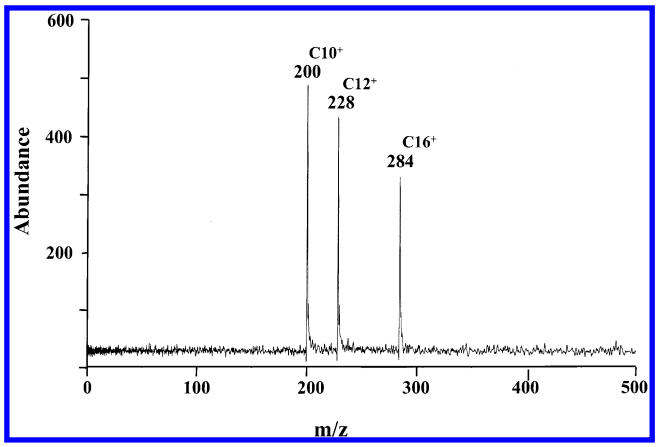

Figure 4. Laser desorption/ionization mass spectrum of the mixture with equal amounts (10 ng) of decyltrimethylammonium bromide, dodecyltrimethylammonium bromide, and tetradecyltrimethylammonium bromide desorbed from the sol−gel-derived DHB (10 000 ppm) film.

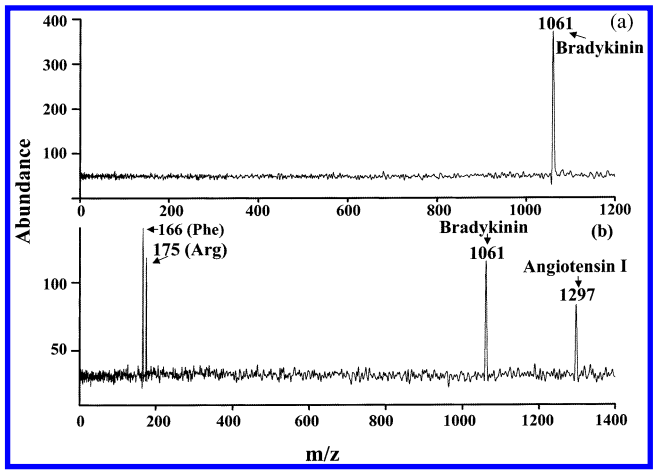

Figure 5. Laser desorption/ionization mass spectra of (a) bradykinin (MW 1060.2, 1 ng) and (b) the mixture with equal amounts (1 ng) of phenylalanine (MW 165.2), arginine (174.2), bradykinin (MW 1060.2), and angiotensin I (MW 1296.5) desorbed from the sol−gel-derived DHB (10 000 ppm) film.

and hexatrimethylammonium bromide directly desorbed form the sol−gel-derived DHB (10 000 ppm) film. The debrominated cationic surfactant signals appear at $m/z = 200$ (C_{10}^+), 228 (C_{12}^+), and 284 (C_{16}^+). This approach takes advantage of a matrix interference-free background and is very suited to analyze low-mass analytes.

This method identifies biomolecules such as amino acids and peptides, as well as small organics. Figure 5a shows the laser desorption/ionization mass spectrum of bradykinin desorbed from the sol−gel-derived DHB (10 000 ppm) film. The only peak in the mass spectrum corresponds to the protonated quasimolecular ion of bradykinin. Furthermore, the analyte signal was found everywhere on the sol−gel-derived DHB film during laser irradiation, indicating that the analyte molecules were evenly distributed on the film by using this sample preparation of SGALDI. Figure 5b shows the laser desorption/ionization mass spectrum of the mixture with equal amounts (1 ng) of phenylalanine (MW 165.2), arginine (MW 174.2), bradykinin (MW 1060.2), and angiotensin I (MW 1296.5). The protonated analyte quasuimolecular ions can be easily identified in the mass spectra with such a clean background.

Figure 6a shows the laser desorption/ionization mass spectra of insulin (1.75 pmol, MW 5733.5) directly desorbed from the sol−gel-derived DHB (10 000 ppm) film. A higher laser power was required to desorb insulin than was required to analyze peptides and small organics. This mass spectrum also shows the dimer, trimer, and tetrameter molecular ions of insulin. Interestingly, during the first laser shot, no matrix ions derived from DHB molecules were observed, except the dominant protonated quasimolecular ions of insulin. However, subsequent laser shots on the same film, even those irradiating different locations, generated

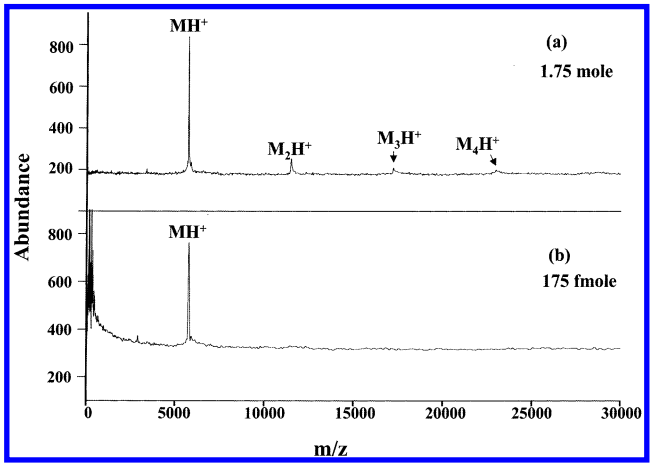

Figure 6. Laser desorption/ionization mass spectra of (a) 10 and (b) 1 ng of insulin (MW 5733.5) desorbed from the sol−gel-derived DHB (10 000 ppm) film.

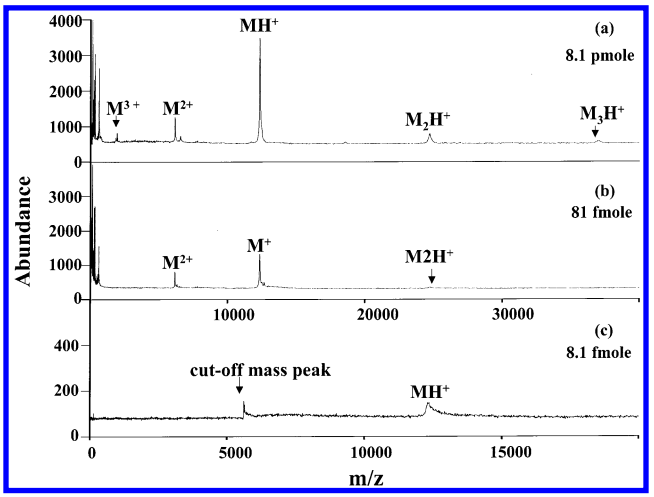

Figure 7. Laser desorption/ionization mass spectra of (a) 8.1 pmol, (b) 81 fmol, and (c) 8.1 fmol of cytochrome *c* desorbed from the sol−gel-derived DHB (10 000 ppm) film.

the DHB-derived matrix ions, along with the protonated quasimolecular ions of insulin. (See Figure 6b.) We suspected that the entire structure of the film might have become slack in response to the first laser shot. However, such slackening did not occur during the analysis of small organics. A higher laser power (>110 μJ) than normally used was generally required to desorb larger molecules, such as in the analysis of these small proteins. Nevertheless, we have not yet been able to interpret this observation satisfactorily.

Figure 7a shows the laser desorption/ionization mass spectrum of cytochrome *c* (8.1 pmol, MW 12 360), obtained from the sol−gel-derived DHB (10 000 ppm) film. In addition to the monomer quasimolecular ion, this mass spectrum shows the dimer (M_2H^+), trimer (M_3H^+), and doubly charged (M^{2+}) ions of cytochrome *c*. Figure 7b shows the mass spectrum of cytochrome *c* at a lower concentration of 81 fmol. The signal associated with monomeric quasimolecular ions is weaker than that in Figure 7a. Moreover, the trimer quasimolecular ion of cytochrome *c* is absent from this mass spectrum. This observation reveals that the ion intensity may depend on the concentrations of analytes. A higher concentration of analyte corresponds to higher analyte ion intensity. The laser power required (>110 μJ) to obtain mass spectra for analyzing proteins was higher than that to analyze small organics. The matrix ions derived from the DHB were also obtained during laser irradiation. Thus, the appearance of the matrix background became unavoidable when larger analytes were analyzed. Nevertheless, this method remains suited to analyze either low-mass organics or protein molecules because the matrix interference in the low-mass region causes no confusion in identifying larger molecules in the mass spectra. The detection limit could be lowered to 8.1 fmol, using a mass spectrometer with a "cutoff-mass" function (Figure 7c). The detection limit of cytochrome *c* in SGALDI analysis is ~10 times higher than that in MALDI analysis (0.81 fmol), according to our experiments. However, the detection limit of insulin in SGALDI analysis is 9 fmol, which is ~5 times higher than that in MALDI analysis (1.8 fmol). The largest analyte yet obtained is myoglobin (MW 16 952.5, result not shown).

An interesting feature of the SGALDI-MS observed here was that the protonated pseudomolecular ions dominated all the mass spectra. The sodium and potassium adduct ions of the analytes, commonly observed in conventional MALDI, were not observed in this study, which fact raised an interesting question about the source of protons. In addition to the two hydroxyl groups, the carboxylic group in the DHB molecule is supposed to be a very weak binding site with the products of hydrolyzing TEOS, because of its withdrawing-electron characteristics. Accordingly, the carboxylic group in the DHB molecules may be free of any binding, and the hydrate ion may be released into the trace of water on the wall of the porously polymeric structure. Consequently, when the laser irradiated the sol−gel-derived DHB film, the H_3O^+ and the analytes may be simultaneously desorbed and the analyte molecules may be protonated in the gas phase via ion−molecule reactions.

Approximately 51−69 TEOS molecules were incorporated with 1 DHB molecule, according to the calculation for the 7500−10 000 ppm DHB entrapped in the sol−gel-derived film. Adding excess DHB to the sol−gel solution could cause free DHB molecules to be present in the sol−gel-derived film, due to the limited number of available binding sites for DHB. Using water to wash off excess DHB molecules from the sol−gel-derived DHB film was not effective. Such a "cleaning step" could crack the sol−gel-derived DHB film. Matrix ions derived from DHB molecules were very easily generated on the cleft when the laser irradiated the cracked film. Thus, controlling the amount of dopant in the sol−gel film is preferred to create a matrix interference-free background. Additionally, entrapping smaller sized dopant compounds with DHB-like properties in the sol−gel-derived film to increase the total number of absorbers may improve the desorption/ionization efficiency of high-mass analytes in SGALDI analysis.

CONCLUSIONS

For the first time, a sol−gel derivative was used as a MALDI matrix. The use of a sol−gel-derived DHB film in analyzing small organics and proteins in laser desorption/ionization mass analysis was demonstrated. A matrix interference-free background was obtained to analyze low-mass analytes on the sol−gel-derived-DHB (7500~10 000 ppm) film during laser irradiation. The detection limit of this method is approximately 5−10 times higher than that of conventional MALDI. Analyte signals were homogeneously

obtained from the sol−gel-derived DHB film. Since many MALDI matrixes have been widely used in conventional MALDI analysis, several of them may also be effective dopants for the sol−gel-derived film, meeting the requirements of the SGALDI-MS analysis. A good dopant must contain at least two binding sites to bond to the silane structure plus an acidic group as proton donor, according to the experimental results presented here. The mass range may be then extended if the dopant also possesses high absorption capacity toward the laser energy using in laser desorption besides the requirements described above. This study is just the beginning of using sol−gel materials to prepare the MALDI samples. Taking advantage of a low matrix background, various methods of analysis, such as capillary electrophoresis, may well be coupled with SGALDI-MS without too much compromise in combination.

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